=> d his

(FILE 'HOME' ENTERED AT 18:11:51 ON 13 NOV 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 18:16:23 ON 13 NOV 2003

L1 2259 S HYPOXIA(3A)RESPONSE(3A)ELEMENT OR HRE
L2 194 S L1(8A)(REPEAT OR TEO OR THREE OR FOUR)
L3 255 S L1(8A)(REPEAT OR TWO OR THREE OR FOUR)
L4 203 S L1(5A)(REPEAT OR TWO OR THREE OR FOUR)
L5 1226 S (HIF OR HYPOXIA-INDUCIBLE(W)FACTOR)(6A)BIND?
L6 14 S L4 AND L5
L7 19 S L3 AND L5
L8 8 DUP REM L7 (11 DUPLICATES REMOVED)
L9 2 S SPACER AND L3
L10 141 S L3 AND (PROMOTER OR PROMOTOR)
L11 80 S L3(S)(PROMOTER OR PROMOTOR)
L12 2 DUP REM L9 (0 DUPLICATES REMOVED)
L13 44 DUP REM L11 (36 DUPLICATES REMOVED)
L14 19 S HYPOXIA(3A)RESPONSE(3A)ELEMENT(8A)(REPEAT OR TWO OR THREE OR
L15 7 DUP REM L14 (12 DUPLICATES REMOVED)
L16 266 S (HYPOXIA(3A)RESPONSE(3A)ELEMENT OR HRE)(8A)(REPEAT OR TWO OR
L17 49 S L16 AND HYPOXIA
L18 18 DUP REM L17 (31 DUPLICATES REMOVED)

=> d au ti so 1-18 l18

L18 ANSWER 1 OF 18 MEDLINE on STN DUPLICATE 1
AU Haque Muzammel; Davis David A; Wang Victoria; Widmer Isabelle; Yarchoan Robert
TI Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) contains **hypoxia** response elements: relevance to lytic induction by **hypoxia**.
SO JOURNAL OF VIROLOGY, (2003 Jun) 77 (12) 6761-8.
Journal code: 0113724. ISSN: 0022-538X.

L18 ANSWER 2 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AU Nelson, Daniel W. [Reprint Author]; Sunar-Reeder, Bulbin [Reprint Author]; Zhu, Yonghua [Reprint Author]; Giaccia, Amato J. [Reprint Author]; Koong, Albert C. [Reprint Author]; Le, Quynh-Thu [Reprint Author]
TI Assessing tumor **hypoxia** by non-invasive urinary secreted marker in mouse xenografts.
SO Proceedings of the American Association for Cancer Research Annual Meeting, (July 2003) Vol. 44, pp. 376. print.
Meeting Info.: 94th Annual Meeting of the American Association for Cancer Research. Washington, DC, USA. July 11-14, 2003.
ISSN: 0197-016X.

L18 ANSWER 3 OF 18 MEDLINE on STN DUPLICATE 2
AU Rapisarda Annamaria; Uranchimeg Badarch; Scudiero Dominic A; Selby Mike; Sausville Edward A; Shoemaker Robert H; Melillo Giovanni
TI Identification of small molecule inhibitors of **hypoxia**-inducible factor 1 transcriptional activation pathway.
SO CANCER RESEARCH, (2002 Aug 1) 62 (15) 4316-24.
Journal code: 2984705R. ISSN: 0008-5472.

L18 ANSWER 4 OF 18 MEDLINE on STN DUPLICATE 3
AU Lu Shan; Gu Xiang; Hoestje Sara; Epner Daniel E
TI Identification of an additional **hypoxia** responsive element in the glyceraldehyde-3-phosphate dehydrogenase gene promoter.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (2002 Mar 19) 1574 (2) 152-6.
Journal code: 0217513. ISSN: 0006-3002.

L18 ANSWER 5 OF 18 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AU Lu S; Gu X; Hoestje S; Epner D E (Reprint)
TI Identification of an additional **hypoxia** responsive element in the glyceraldehyde-3-phosphate dehydrogenase gene promoter
SO BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (19 MAR 2002) Vol. 1574, No. 2, pp. 152-156.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0167-4781.

L18 ANSWER 6 OF 18 MEDLINE on STN DUPLICATE 4
AU Charlier Nico; Leclere Norbert; Felderhoff Ursula; Heldt Julia; Kietzmann Thomas; Obladen Michael; Gross Johann
TI **Hypoxia**-induced cell death and changes in **hypoxia** -inducible factor-1 activity in PC12 cells upon exposure to nerve growth factor.
SO BRAIN RESEARCH. MOLECULAR BRAIN RESEARCH, (2002 Jul 15) 104 (1) 21-30.
Journal code: 8908640. ISSN: 0169-328X.

L18 ANSWER 7 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AU Lis, A. [Reprint Author]; Garrick, M. D. [Reprint Author]; Roth, J. A. [Reprint Author]
TI **HYPOXIA** INDUCES CHANGES IN THE EXPRESSION AND SUBCELLULAR DISTRIBUTION OF THE ISOFORMS OF DIVALENT METAL TRANSPORTER (DMT1) IN RAT PHEOCHROMOCYTOMA (PC12) CELLS.
SO Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002) Vol. 2002, pp. Abstract No. 100.4. http://sfn.scholarone.com. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience. Orlando, Florida, USA. November 02-07, 2002. Society for Neuroscience.

L18 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2003 ACS on STN
IN Webster, Keith A.
TI Combinations of silencer and inducible regulatory elements for tight regulation and strong induction of foreign genes in animal cells
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2

L18 ANSWER 9 OF 18 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AU Qin C H; Wilson C; Blancher C; Taylor M; Safe S; Harris A L (Reprint)
TI Association of ARNT splice variants with estrogen receptor-negative breast cancer, poor induction of vascular endothelial growth factor under **hypoxia**, and poor prognosis
SO CLINICAL CANCER RESEARCH, (APR 2001) Vol. 7, No. 4, pp. 818-823.
Publisher: AMER ASSOC CANCER RESEARCH, PO BOX 11806, BIRMINGHAM, AL 35202 USA.
ISSN: 1078-0432.

L18 ANSWER 10 OF 18 CAPLUS COPYRIGHT 2003 ACS on STN
IN Binley, Katie Mary; Naylor, Stuart
TI A **hypoxia**-responsive regulatory element and its use in the expression of therapeutic genes in chronically hypoxic solid tumors
SO PCT Int. Appl., 154 pp.
CODEN: PIXXD2

L18 ANSWER 11 OF 18 MEDLINE on STN DUPLICATE 5
AU Mukhopadhyay C K; Mazumder B; Fox P L
TI Role of **hypoxia**-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jul 14) 275 (28) 21048-54.
Journal code: 2985121R. ISSN: 0021-9258.
(Investigators: Fox P L, Cleveland Clinic Found, OH)

L18 ANSWER 12 OF 18 MEDLINE on STN DUPLICATE 6
AU Sugawara J; Tazuke S I; Suen L F; Powell D R; Kaper F; Giaccia A J;

Giudice L C
TI Regulation of insulin-like growth factor-binding protein 1 by **hypoxia** and 3',5'-cyclic adenosine monophosphate is additive in HepG2 cells.
SO JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, (2000 Oct) 85 (10) 3821-7.
Journal code: 0375362. ISSN: 0021-972X.

L18 ANSWER 13 OF 18 MEDLINE on STN DUPLICATE 7
AU Sugawara J; Suh D S; Faessen G H; Suen L F; Shibata T; Kaper F; Giaccia A J; Giudice L C
TI Regulation of insulin-like growth factor-binding protein-1 by nitric oxide under hypoxic conditions.
SO JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, (2000 Aug) 85 (8) 2714-21.
Journal code: 0375362. ISSN: 0021-972X.

L18 ANSWER 14 OF 18 MEDLINE on STN DUPLICATE 8
AU Shibata T; Giaccia A J; Brown J M
TI Development of a **hypoxia**-responsive vector for tumor-specific gene therapy.
SO GENE THERAPY, (2000 Mar) 7 (6) 493-8.
Journal code: 9421525. ISSN: 0969-7128.

L18 ANSWER 15 OF 18 MEDLINE on STN DUPLICATE 9
AU Ruan H; Wang J; Hu L; Lin C S; Lamborn K R; Deen D F
TI Killing of brain tumor cells by **hypoxia**-responsive element mediated expression of BAX.
SO NEOPLASIA, (1999 Nov) 1 (5) 431-7.
Journal code: 100886622. ISSN: 1522-8002.

L18 ANSWER 16 OF 18 MEDLINE on STN DUPLICATE 10
AU Tazuke S I; Mazure N M; Sugawara J; Carland G; Faessen G H; Suen L F; Irwin J C; Powell D R; Giaccia A J; Giudice L C
TI **Hypoxia** stimulates insulin-like growth factor binding protein 1 (IGFBP-1) gene expression in HepG2 cells: a possible model for IGFBP-1 expression in fetal **hypoxia**.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Aug 18) 95 (17) 10188-93.
Journal code: 7505876. ISSN: 0027-8424.

L18 ANSWER 17 OF 18 MEDLINE on STN DUPLICATE 11
AU Sogawa K; Numayama-Tsuruta K; Ema M; Abe M; Abe H; Fujii-Kuriyama Y
TI Inhibition of **hypoxia**-inducible factor 1 activity by nitric oxide donors in **hypoxia**.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Jun 23) 95 (13) 7368-73.
Journal code: 7505876. ISSN: 0027-8424.

L18 ANSWER 18 OF 18 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AU Semenza G L (Reprint); Jiang B H; Leung S W; Passantino R; Concordet J P; Maire P; Giallongo A
TI **Hypoxia** response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for **hypoxia**-inducible factor 1
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (20 DEC 1996) Vol. 271, No. 51, pp. 32529-32537.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.

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=> d his

(FILE 'HOME' ENTERED AT 18:11:51 ON 13 NOV 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 18:16:23 ON 13 NOV 2003

L1 2259 S HYPOXIA(3A)RESPONSE(3A)ELEMENT OR HRE
L2 194 S L1(8A)(REPEAT OR TEO OR THREE OR FOUR)
L3 255 S L1(8A)(REPEAT OR TWO OR THREE OR FOUR)
L4 203 S L1(5A)(REPEAT OR TWO OR THREE OR FOUR)
L5 1226 S (HIF OR HYPOXIA-INDUCIBLE(W)FACTOR)(6A)BIND?
L6 14 S L4 AND L5
L7 19 S L3 AND L5
L8 8 DUP REM L7 (11 DUPLICATES REMOVED)

=> d bib ab 1-8 l8

L8 ANSWER 1 OF 8 MEDLINE on STN DUPLICATE 1
AN 2003245120 MEDLINE
DN 22652788 PubMed ID: 12767996
TI Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) contains hypoxia response elements: relevance to lytic induction by hypoxia.
AU Haque Muzammel; Davis David A; Wang Victoria; Widmer Isabelle; Yarchoan Robert
CS HIV and AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892-1868, USA.
SO JOURNAL OF VIROLOGY, (2003 Jun) 77 (12) 6761-8.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200307
ED Entered STN: 20030528
Last Updated on STN: 20030704
Entered Medline: 20030703
AB Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), also known as human herpesvirus 8, is an etiologic agent of KS, primary effusion lymphoma (PEL), and multicentric Castleman's disease. We recently demonstrated that hypoxia can induce lytic replication of KSHV in PEL cell lines. Hypoxia induces the accumulation of hypoxia-inducible factors (HIF), and we hypothesized that the KSHV genome may respond to hypoxia through functional hypoxia response elements (HREs). Here, we demonstrate the presence of at least two promoters within the KSHV genome that are activated by hypoxia or hypoxia mimics. One is in the promoter region of the gene for Rta, the main lytic switch gene, and the other is within the promoter region of ORF34, a lytic gene of unknown function. The ORF34 promoter contains **three** putative consensus **HREs** oriented in the direction of the gene. Dissection and site-directed mutagenesis studies confirmed that one of the HREs of the ORF34 promoter is functional. Under conditions of hypoxia, the ORF34 promoter was strongly upregulated by HIF-1 alpha and HIF-2 alpha. By contrast, the promoter of the gene for Rta appeared to be preferentially upregulated by HIF-2 alpha. Reverse transcription-PCR analysis revealed that specific messages for ORF34 and ORF50 are upregulated in BCBL-1 cells exposed to hypoxia. An **HIF**-1 **binding** and competition assay demonstrated that the HRE sequence from the ORF34 promoter can compete for **HIF**-1 alpha **binding** to an erythropoietin HRE oligonucleotide while a mutant sequence cannot. Thus, we demonstrated that a viral gene can be activated by hypoxia through activation of a functional viral HRE. To our knowledge, this is the first example of a functional HRE in a viral promoter.

L8 ANSWER 2 OF 8 MEDLINE on STN DUPLICATE 2
AN 2002219088 MEDLINE
DN 21952369 PubMed ID: 11955624
TI Identification of an additional hypoxia responsive element in the glyceraldehyde-3-phosphate dehydrogenase gene promoter.
AU Lu Shan; Gu Xiang; Hoestje Sara; Epner Daniel E
CS Department of Medicine, Baylor College of Medicine, VA Medical Center, Houston, TX 77030, USA.
NC R29 CA78355 (NCI)
SO BIOCHIMICA ET BIOPHYSICA ACTA, (2002 Mar 19) 1574 (2) 152-6.
Journal code: 0217513. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200206
ED Entered STN: 20020417
Last Updated on STN: 20020628
Entered Medline: 20020627
AB Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multifunctional enzyme overexpressed in many tumors and induced by hypoxia in normal and malignant cells. The degree to which hypoxia transcriptionally activates GAPDH is cell type specific. The GAPDH promoter region contains a hypoxia responsive element (HRE) consisting of a **hypoxia inducible factor**-1 (**HIF**-1) consensus **binding** site plus adjacent sequence [Graven et al. (1999) Biochim. Biophys. Acta 1447, 208-218]. Using transient transfection experiments with the GAPDH promoter region linked to a luciferase reporter gene, we found that GAPDH was transcriptionally activated by hypoxia in each of three human prostate cancer cell lines tested, with the greatest level of induction in the most differentiated cell line. Using sequence analysis of the GAPDH promoter region, we identified a novel HRE distinct from the previously characterized one that consists of two consensus HIF-1 sites arranged as inverted repeats separated by 5 bp. Hypoxia transcriptionally activated a promoter construct in which the previously characterized HRE was mutated and the novel HRE remained intact. Heterologous promoter constructs containing only one or **two** copies of the novel **HRE** plus a minimal promoter consisting of a TATA box drove hypoxia inducible expression of the luciferase reporter gene in transient transfection assays. Mutation of HIF-1 sites within the novel HRE resulted in complete loss of function.

L8 ANSWER 3 OF 8 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
AN 2002:372214 SCISEARCH
GA The Genuine Article (R) Number: 547EX
TI Identification of an additional hypoxia responsive element in the glyceraldehyde-3-phosphate dehydrogenase gene promoter
AU Lu S; Gu X; Hoestje S; Epner D E (Reprint)
CS Baylor Coll Med, VA Med Ctr, Dept Med, Houston, TX 77030 USA (Reprint)
CYA USA
SO BIOCHIMICA ET BIOPHYSICA ACTA-GENE STRUCTURE AND EXPRESSION, (19 MAR 2002) Vol. 1574, No. 2, pp. 152-156.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0167-4781.
DT Article; Journal
LA English
REC Reference Count: 24
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a multifunctional enzyme overexpressed in many tumors and induced by hypoxia in normal and malignant cells. The degree to which hypoxia transcriptionally activates GAPDH is cell type specific. The GAPDH promoter region contains a hypoxia responsive element (HRE) consisting of a **hypoxia**

inducible factor-1 (**HIF**-1) consensus **binding** site plus adjacent sequence [Graven et al. (1999) Biochim. Biophys. Acta 1447, 208-218]. Using transient transfection experiments with the GAPDH promoter region linked to a luciferase reporter gene, we found that GAPDH was transcriptionally activated by hypoxia in each of three human prostate cancer cell lines tested, with the greatest level of induction in the most differentiated cell line. Using sequence analysis of the GAPDH promoter region, we identified a novel HRE distinct from the previously characterized one that consists of two consensus HIF-1 sites arranged as inverted repeats separated by 5 bp. Hypoxia transcriptionally activated a promoter construct in which the previously characterized HRE was mutated and the novel HRE remained intact. Heterologous promoter constructs containing only one or **two** copies of the novel **HRE** plus a minimal promoter consisting of a TATA box drove hypoxia inducible expression of the luciferase reporter gene in transient transfection assays. Mutation of HIF-1 sites within the novel HRE resulted in complete loss of function. (C) 2002 Elsevier Science B.V. All rights reserved.

L8 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2001:489616 CAPLUS
DN 135:88021
TI Combinations of silencer and inducible regulatory elements for tight regulation and strong induction of foreign genes in animal cells
IN Webster, Keith A.
PA University of Miami, USA
SO PCT Int. Appl., 48 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001048187	A2	20010705	WO 2000-US33269	20001215
	WO 2001048187	A3	20020530		
	WO 2001048187	C2	20021107		
	W: CA, JP				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
	EP 1242592	A2	20020925	EP 2000-984041	20001215
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	JP 2003523182	T2	20030805	JP 2001-548700	20001215
PRAI	US 1999-171597P	P	19991223		
	US 2000-723326	A	20001128		
	WO 2000-US33269	W	20001215		

AB Expression vectors are disclosed that are comprised of one or more silencer elements and conditionally inducible elements to form silencer-inducible regions and promoters in operative linkage upstream of at least one expressed region. The expression vector thereby regulates expression of at least one downstream region by conditional silencing in which an expressed DNA region of a gene is transcribed. Use of multiple copies of the silencer lowers the basal level of expression of the gene and therefore increases the induction ratio. Genetically engineered mammalian cells and non-human mammals can be made using such expression vectors through transfection and transgenesis techniques. Moreover, processes of making and using the aforementioned products are disclosed (e.g., the expression vector may be used diagnostically, therapeutically, or prophylactically). A series of constructs using repeats of the silencer element (SIL) of the human synapsin gene and the hypoxia response element (HRE) of the phosphoglycerate kinase gene were prepd. and used to regulate expression of a luciferase reporter gene from the SV40 early promoter in animal cells. Induction of the reporter gene in hypoxic skeletal myocytes was directly proportional to the no. of copies of

SIL/HRE pairs in the promoter region. The construct was more effective in skeletal myocytes than in cardiac myocytes. In a rat ischemic hindlimb model induction ratios for the reporter gene under ischemic (hypoxic) conditions was >20 for constructs carrying **three** copies of the SIL/**HRE** pairs. For animals carrying only **three** copies of the **HRE** element and no silence elements the induction ratio was .apprx.1.4.

L8 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2000:210397 CAPLUS
DN 132:247196
TI A hypoxia-responsive regulatory element and its use in the expression of therapeutic genes in chronically hypoxic solid tumors
IN Binley, Katie Mary; Naylor, Stuart
PA Oxford Biomedica (UK) Limited, UK
SO PCT Int. Appl., 154 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000017371	A1	20000330	WO 1999-GB3181	19990922
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	WO 9915684	A2	19990401	WO 1998-GB2885	19980923
	WO 9915684	A3	19990610		
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2343324	AA	20000330	CA 1999-2343324	19990922
	AU 9962130	A1	20000410	AU 1999-62130	19990922
	EP 1115877	A1	20010718	EP 1999-949142	19990922
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002526083	T2	20020820	JP 2000-574270	19990922
PRAI	WO 1998-GB2885	W	19980923		
	GB 1999-1906	A	19990128		
	GB 1999-3538	A	19990216		
	GB 1997-20216	A	19970923		
	GB 1997-20465	A	19970925		
	WO 1999-GB3181	W	19990922		

AB A polynucleotide is provided which comprises at least **two repeats** of a **hypoxia response element** (**HRE**), wherein the **hypoxia-inducible factor** consensus **binding** sites within each of the two repeats are sepd. by a spacer of at least 20 contiguous nucleotides. A polynucleotide is also provides which comprised at least **three repeats** of a phosphoglycerate kinase (PGK) **hypoxia response element** (**HRE**) operably linked to an SV40 promoter or an MLV promoter. The polynucleotide may be operably linked to a nucleic acid of interest to drive expression under hypoxic

conditions. In particular, the element can be used to drive expression of therapeutic genes in solid tumors as they are often undervascularized and chronically hypoxic. A series of HREs from a no. of different hypoxia-inducible genes were tested for hypoxia inducibility and those showing the strongest induction were selected for further characterization and optimization. The construct derived from the PGK gene, showing induction ratios of .gtoreq.100, was found to contain a consensus **binding** sequence for **hypoxia-inducible factor** (**HIF**) and these constructs could confer hypoxia inducible expression on a gene in macrophages. Although the construct contained the HIF-responsive element, no HIF could be detected, but macrophages manuf. the closely related EPAS-1 (endothelial PAS domain protein 1) transcription factor. The HRE could be used in combination with retroviral promoters. Gene therapy constructs using the HRE in combination with therapeutic genes, e.g. for a prodrug-activating cytochrome P 450, or tissue-specific regulatory elements such as interferon response elements are described.

RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 3
AN 2000396648 MEDLINE
DN 20347210 PubMed ID: 10777486
TI Role of hypoxia-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency.
AU Mukhopadhyay C K; Mazumder B; Fox P L
CS Department of Cell Biology, The Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA.
NC HL29582 (NHLBI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jul 14) 275 (28) 21048-54.
Journal code: 2985121R. ISSN: 0021-9258.
(Investigators: Fox P L, Cleveland Clinic Found, OH)
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Space Life Sciences
EM 200008
ED Entered STN: 20000824
Last Updated on STN: 20000824
Entered Medline: 20000816
AB A role of the copper protein ceruloplasmin (Cp) in iron metabolism is suggested by its ferroxidase activity and by the tissue iron overload in hereditary Cp deficiency patients. In addition, plasma Cp increases markedly in several conditions of anemia, e.g. iron deficiency, hemorrhage, renal failure, sickle cell disease, pregnancy, and inflammation. However, little is known about the cellular and molecular mechanism(s) involved. We have reported that iron chelators increase Cp mRNA expression and protein synthesis in human hepatocarcinoma HepG2 cells. Furthermore, we have shown that the increase in Cp mRNA is due to increased rate of transcription. We here report the results of new studies designed to elucidate the molecular mechanism underlying transcriptional activation of Cp by iron deficiency. The 5'-flanking region of the Cp gene was cloned from a human genomic library. A 4774-base pair segment of the Cp promoter/enhancer driving a luciferase reporter was transfected into HepG2 or Hep3B cells. Iron deficiency or hypoxia increased luciferase activity by 5-10-fold compared with untreated cells. Examination of the sequence showed **three** pairs of consensus hypoxia-responsive elements (**HREs**). Deletion and mutation analysis showed that a single HRE was necessary and sufficient for gene activation. The involvement of hypoxia-inducible factor-1 (HIF-1) was shown by gel-shift and supershift experiments that showed **HIF**-1alpha and **HIF**-1beta **binding** to a radiolabeled oligonucleotide containing the Cp promoter HRE. Furthermore, iron deficiency (and hypoxia) did not activate Cp gene expression in Hepa

c4 hepatoma cells deficient in HIF-1beta, as shown functionally by the inactivity of a transfected Cp promoter-luciferase construct and by the failure of **HIF**-1 to **bind** the Cp HRE in nuclear extracts from these cells. These results are consistent with in vivo findings that iron deficiency increases plasma Cp and provides a molecular mechanism that may help to understand these observations.

L8 ANSWER 7 OF 8 MEDLINE on STN DUPLICATE 4
AN 1998301580 MEDLINE
DN 98301580 PubMed ID: 9636155
TI Inhibition of hypoxia-inducible factor 1 activity by nitric oxide donors in hypoxia.
AU Sogawa K; Numayama-Tsuruta K; Ema M; Abe M; Abe H; Fujii-Kuriyama Y
CS Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-77, Japan.. sogawa@mail.cc.tohoku.ac.jp
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Jun 23) 95 (13) 7368-73.
Journal code: 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199808
ED Entered STN: 19980817
Last Updated on STN: 19980817
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AB Nitric oxide (NO) is known to have various biologic and pathophysiologic effects on organisms. The molecular mechanisms by which NO exerts harmful effects are unknown, although various O2 radicals and ions that result from reactivity of NO are presumed to be involved. Here we report that adaptive cellular response controlled by the transcription factor hypoxia-inducible factor 1 (HIF-1) in hypoxia is suppressed by NO. Induction of erythropoietin and glycolytic aldolase A mRNAs in hypoxically cultured Hep3B cells, a human hepatoma cell line, was completely and partially inhibited, respectively, by the addition of sodium nitroprusside (SNP), which spontaneously releases NO. A reporter plasmid carrying **four hypoxia-response element** sequences connected to the luciferase structural gene was constructed and transfected into Hep3B cells. Inducibly expressed luciferase activity in hypoxia was inhibited by the addition of SNP and two other structurally different NO donors, S-nitroso-L-glutathione and 3-morpholinosydnonimine, giving IC50 values of 7.8, 211, and 490 microM, respectively. Inhibition by SNP was also observed in Neuro 2A and HeLa cells, indicating that the inhibition was not cell-type-specific. The vascular endothelial growth factor promoter activity that is controlled by HIF-1 was also inhibited by SNP (IC50 = 6.6 microM). Induction generated by the addition of cobalt ion (this treatment mimics hypoxia) was also inhibited by SNP (IC50 = 2.5 microM). Increased luciferase activity expressed by cotransfection of effector plasmids for HIF-1alpha or HIF-1alpha-like factor in hypoxia was also inhibited by the NO donor. We also showed that the inhibition was performed by blocking an activation step of **HIF**-1alpha to a DNA-**binding** form.

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TI Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential **binding** sites for **hypoxia-inducible factor** 1
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ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Hypoxia-inducible factor 1 (HIF-1) is a basic helix-loop-helix transcription factor which is expressed when mammalian cells are subjected to hypoxia and which activates transcription of genes encoding erythropoietin, vascular endothelial growth factor, and other pro teins that are important for maintaining oxygen homeostasis. Previous studies have provided indirect evidence that HIF-1 also regulates transcription of genes encoding glycolytic enzymes, In this paper we characterize hypoxia response elements in the promoters of the ALDA, ENO1, and Ldha genes, We demonstrate that HIF-1 plays an essential role in activating transcription via these elements and show that although absolutely necessary, the presence of a **HIF**-1 **binding** site alone is not sufficient to mediate transcriptional responses to hypoxia, Analysis of hypoxia response elements in the ENO1 and Ldha gene promoters revealed that each contains two functionally-essential HIF-1 sites arranged as direct and inverted **repeats**, respectively. Our data establish that functional **hypoxia-response elements** consist of a pair of contiguous transcription factor binding sites at least one of which contains the core sequence 5'-RCGTG-3' and is recognized by HIF-1. These results provide further evidence that the coordinate transcriptional activation of genes encoding glycolytic enzymes which occurs in hypoxic cells is mediated by HIF-1.

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